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(54) Title: DESENSITIZATION TO SPECIFIC ALLERGENS

(57) Abstract

A method for desensitizing an animal to a particular allergen, wherein at or about a time of exposure of the animal to the allergen, a molecule is administered to the animal, which molecule is characterized in that it specifically binds under physiological conditions to an interleukin-4 (IL-4) receptor expressed on a peripheral blood mononuclear cell (PBMC) of the animal, and is capable of decreasing the viability of the PBMC to which it binds.

DESENSITIZATION TO SPECIFIC ALLERGENS

Background of the Invention

The field of the invention is prevention and treatment of allergies.

An allergy is an immunological reaction, generally of the immediate hypersensitivity type, to a particular type of antigen termed an allergen. Such reactions underlie attacks of anaphylaxis, allerigic rhinitis (hay fever), hives, and allergic asthma, and may be triggered by common allergens such as ragweed, pollen, bee or wasp venom, animal dander, mold, or a component of house dust (such as mites). In humans, immediate hypersensitivity (IH) is mediated by antibodies of the IgE isotype anchored to the surfaces of mast cells and basophils in the skin and elsewhere. Binding of antigen to these cell-bound IgE molecules triggers release of mediators such as histamine from the cells, which mediators induce the clinical phenomena such as tissue swelling, itching, or bronchial smooth muscle contraction that typify an allergic reaction.

IgE antibodies specific for a given allergen are produced and secreted by B lymphocytes upon contact with that allergen. Initially, B lymphocytes (or B cells) express antibodies of the IgM isotype, with each B cell committed to producing antibody specific for a particular antigenic determinant. Contact with both an allergen bearing that antigenic determinant, and certain factors produced by T lymphocytes, will induce the B cell to undergo what is termed an antibody heavy chain class switch, in which the antigen-specific portion of the antibody produced by the B cell remains the same, but it is attached to ϵ-heavy chain (to yield IgE antibody) rather than the μ-heavy chain of the IgM isotype. Such a class switch is apparently permanent for a given B cell,

which thereafter secretes IgE antibody specific for the allergen whenever stimulated to do so. One of the factors which has been shown to be involved in this class switch event is interleukin-4 (IL-4) (Lebman and Coffman, *J. Exp. Med.* 168:853, 1988), a 20kD protein produced by T lymphocytes. Human IL-4 has been cloned and sequenced by Yodota et al. (*Proc. Natl. Acad. Sci. USA 83: 58994, 1986*).

Common treatments for allergy include avoidance of the suspected allergen; injections of the allergen as immunotherapy to stimulate certain protective mechanisms and thereby eventually desensitize the host to the allergen; drugs such as corticosteroids, which interfere with the release of the mediators of allergy from mast cells; and drugs such as antihistamines, which block the biological action of the released mediators.

Summary of the Invention

It has now been found that, by treating B cells which have not yet undergone the class switch from IgM production to IgE production with a cytotoxic compound (such as the recombinant protein referred to as DAB_{389}IL-4) that binds to IL-4 receptors on such cells, the production of IgE by such cells following exposure to an allergen analog (αCD40 MAb) can be prevented. In contrast, production of IgE by B cells that have previously switched isotypes is only slightly inhibited by treatment with such an IL-4R-targeting cytotoxic compound, and only by very high levels of the compound. It is believed that the mechanism by which this occurs is as follows: those IgM-expressing B cells which are stimulated by allergen to begin the process of class switching first express a temporarily increased number of IL-4 receptors on their surfaces, which render the cells more likely to bind molecules of an IL-4R-targeted

cytotoxin. Like IL-4 itself, the IL-4R-targeted cytotoxin causes the receptor to which it binds to be internalized by the cell, carrying the cytotoxin, along with the receptor to which it is bound, into the vesicle so formed. The cytotoxic portion of the compound then exits the vesicle and enters the cytoplasm of the cell, where it enzymatically inactivates a crucial cellular protein synthesis factor. With protein synthesis shut off, IgE cannot be made, nor will the cell survive long: thus, any B cells induced by an allergen to undergo a class switch are selectively disabled or killed, leaving only previously-switched B cells to produce IgE in response to the allergen. Furthermore, treatment with a cytotoxin that targets receptors such as IL-4R, IL-2R, or IL-6R provides another mechanism of reducing IgE production, by killing or disabling those peripheral blood mononuclear cells (PBMC's) which are activated in the presence of the allergen to produce factors such as cytokines which stimulate IgE production by B cells. The invention therefore features a method for desensitizing an animal to a particular allergen, whereby an animal in need of such desensitization is first identified, and at or about a time of exposure of the animal to the allergen, a molecule is administered to the animal which specifically binds under physiological conditions to an IL-4 receptor expressed on a PBMC (preferably a B cell), of the animal, the molecule being capable of decreasing the viability of the PBMC (i.e., the molecule is inherently able to contribute to the death or temporary disablement of a PBMC to which it binds via an IL-4 receptor): preferably, the molecule, following binding to an IL-4 receptor, kills the PBMC to which it has bound. The "exposure" referred to may be a result of deliberately administering the allergen to the patient (e.g., by injection), or the patient's having

inadvertantly or intentionally come in contact with an environmental source of the allergen outside of a clinical setting (e.g., by inhaling ragweed during hay fever season, by holding a pet the dander of which is allergenic, or by receiving a bee sting). To be effective, the IL-4R-targeted cytotoxin must be administered at or about the time of (i.e., just prior to, contemporaneously with, or soon after) exposure of the subject animal to the allergen of interest, to ensure that most or all of the animal's allergen-stimulated, unswitched B cells and other PBMCs will bear their temporarily heightened levels of IL-4 receptors during the period that the cytotoxin is present in the animal's bloodstream. Administration of the cytotoxin may be continued even after contact with the allergen has ceased, to ensure that all susceptible B cells are ultimately prevented from making the isotype switch, and/or that other IL-4R-bearing PBMCs in the animal are prevented from contributing to the IgE-secretion process. Because at any time new B cells may arise which have the potential of being triggered into switching isotype by the allergen, the treatment is preferably repeated on a regular basis.

The animal is preferably a mammal such as a mouse or a dog, and most preferably is a human patient who is either naive (i.e., has never previously been exposed to the allergen of interest, or at least never in a manner sufficient to trigger an immunogenic response), or is atopic (i.e., has demonstrated an allergic response to this or a related allergen in the past). Thus, the term "desensitization" refers not only to the method as applied to atopic individuals, but also as the method is applied to anyone, even naive subjects, who may be treated prophylactically in order to ensure they never develop an allergy to the given allergen. Such

prophylactic treatment would be of particular benefit to children of atopic parents, who run a greatly increased risk, compared to the children of non-atopic parents, of eventually developing allergies.

In preferred embodiments, the molecule used in the method of the invention is a hybrid molecule (such as a polypeptide) having a first and a second portion joined together covalently, the first portion including a moiety capable of decreasing cell viability and the second portion including a moiety capable of specifically binding to an IL-4 receptor on a PBMC under physiological conditions [i.e., upon contact with such an IL-4 receptor under physiological conditions, the moiety binds to IL-4 receptors and does not bind detectably to any other structure found on the surfaces of PBMC's from the same species]. By "under physiological conditions" is meant in blood or serum, or in an aqueous solution such as phosphate-buffered saline that approximates the pH and salt conditions which occur in blood *in vivo*. The IL-4R-binding moiety may be, for example, IL-4, an IL-4R-binding portion of IL-4, an IL-4R-binding monoclonal antibody, or an IL-4R-binding portion of such a monoclonal antibody. Where the animal to be treated is a human, the moiety is preferably human IL-4.

The "first portion" of the hybrid molecule preferably includes an enzymatically-active segment of a polypeptide toxin such as diphtheria toxin, *Pseudomonas* exotoxin A, ricin, Shiga toxin, Shiga-like toxin-I, Shiga-like toxin II, Shiga-like toxin II_v, *E. coli* LT, *Salmonella* LT, cholera toxin, C3 toxin, pertussis toxin, tetanus toxin, abrin, modeccin, volkensin, viscumin, alorin, saporin, or gelonin, and more preferably includes fragment A of diphtheria toxin and a portion of fragment B of diphtheria toxin, but does not possess a generalized eukaryotic cell-binding activity such as is found on

fragment B of diphtheria toxin and many other toxins. Most preferably, the toxin segment is DAB_{389} or DAB_{486}, and the hybrid molecule is DAB_{389}IL-4 or DAB_{486}IL-4. Treatment with such an IL-4R-binding cytotoxin may optionally be accompanied by administering to the animal a second cytotoxic molecule which specifically binds under physiological conditions to an interleukin-2 (IL-2) or interleukin-6 (IL-6) receptor on the same PBMC as is bound by the IL-4R-binding cytotoxin, or on a different PBMC (monocyte, T cell or B cell). Such ancillary treatment may help minimize the level of IgE production resulting from contact with the allergen.

Also within the invention is a method for inhibiting the antibody heavy-chain class switching of a B cell by treating a B cell which has not yet undergone class switching with a molecule which is capable of specifically binding to an IL-4 receptor expressed on the surface of the B cell, the molecule being capable of decreasing the viability of the B cell (i.e., the molecule possesses the inherent capacity to contribute to the disablement or death of a B cell to which it binds via an IL-4 receptor). This method may be carried out *in vitro*, using a biological sample such as blood or purified B cells, or *in vivo*, such as in a human patient. It is preferentially accompanied by the additional step of contacting the B cell with an allergen at or about the same time as the treatment step (i.e., shortly before, during, or soon after the treatment step). The molecule used may be any of the cytotoxic IL-4R-binding hybrid molecules discussed herein, but is preferably a diphtheria toxin-based recombinant polypeptide such as DAB_{389}IL-4.

By selectively killing or disabling B cells poised to switch to the IgE isotype upon contact with a given allergen, the method of the invention provides an

effective means of desensitizing individuals to that allergen. For those who suffer from allergies, preventing such class switching may result in a gradually decreased allergic response to the allergen as existing IgE and IgE-producing cells are naturally turned over without being replaced by newly-switched B cells. Naive individuals desensitized to a given allergen in accordance with the method of the invention may never develop an allergy to that allergen.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Detailed Description

The drawing is first described.

Drawing

Fig. 1 is a representation of the amino acid sequence of $DAB_{389}IL-4$ and a DNA sequence encoding this hybrid protein (SEQ ID NO: 1).

IL-4-receptor-targeted toxins

The compounds useful in the method of the invention preferably contain toxic moieties, such as bacterial polypeptide toxins or enzymatically-active portions thereof, which are significantly cytotoxic only when present intracellularly. Of course, under these circumstances, the molecule must be able to enter a cell bearing the targeted IL-4 receptor (IL-4R). This may be accomplished by including on the toxin molecule a ligand (such as IL-4 itself, or a portion of IL-4 capable of binding to the IL-4 receptor, or an anti-IL-4R antibody) which, upon binding to the receptor, induces the internalization of the receptor and anything bound to it. Such an IL-4R-binding moiety can be linked to the toxin molecule chemically, using standard chemical conjugation

techniques. Alternatively, the linkage can be accomplished by engineering a hybrid recombinant DNA molecule which encodes both the IL-4R-binding moiety and the toxin in a single polypeptide. The latter approach ensures consistency of composition.

Many peptide toxins have a generalized eukaryotic receptor binding domain; in these instances the toxin must be modified to prevent intoxication of non-IL-4R-bearing cells. Any such modifications must be made in a manner which preserves the cytotoxic functions of the molecule. Potentially useful polypeptide toxins include, but are not limited to: diphtheria toxin, *Pseudomonas* exotoxin A, cholera toxin, ricin, Shiga toxin, the Shiga-like toxins (SLT-I, SLT-II, SLT II_V), *E. coli* LT, *Salmonella* LT, C3 toxin, pertussis toxin, tetanus toxin, abrin, modeccin, volkensin, viscumin, alorin, saporin, and gelonin.

Other types of toxic moieties which may be linked to an IL-4R-binding ligand for use in the method of the invention include, for example, radionuclides and cancer chemotherapeutic agents.

Diphtheria Toxin-based Molecules

Diphtheria toxin, which is described in detail in Murphy U.S. Patent No. 4,675,382 (hereby incorporated by reference), can be used to produce molecules useful in the method of the invention. The natural diphtheria toxin molecule secreted by *Corynebacterium diphtheriae* consists of several functional domains which can be characterized, starting at the amino terminal end of the molecule, as fragment A (amino acids Gly_1 - Arg_{193}), which is the enzymatically-active portion of the protein, and fragment B (amino acids Ser_{194} - Ser_{535}), which includes a translocation domain and a generalized cell binding domain (amino acid residues 475 through 535).

The process by which diphtheria toxin intoxicates sensitive eukaryotic cells involves at least the following steps: (i) the binding domain of diphtheria toxin binds to specific receptors on the surface of a sensitive cell; (ii) while bound to its receptor, the toxin molecule is internalized into an endocytic vesicle; (iii) either prior to internalization, or within the endocytic vesicle, the toxin molecule undergoes a proteolytic cleavage between fragments A and B; (iv) as the pH of the endocytic vesicle decreases to below 6, the toxin crosses the endosomal membrane, facilitating the delivery of fragment A into the cytosol; (v) the catalytic activity of fragment A (i.e., the nicotinamide adenine dinucleotide - dependent adenosine diphosphate (ADP) ribosylation of the eukaryotic protein synthesis factor termed "Elongation Factor 2") causes the death of the intoxicated cell. It has been shown that a single molecule of fragment A introduced into the cytosol is sufficient to shut down the cell's protein synthesis machinery, thereby killing the cell. The mechanism of cell killing by *Pseudomonas* exotoxin A, and possibly by certain other naturally-occurring toxins, is the same.

$DAB_{389}IL-4$, a genetically engineered fusion protein in which the receptor binding domain of diphtheria toxin has been replaced by human IL-4, is an example of a molecule useful in the method of the invention. This molecule selectively kills IL-4R-expressing cells, including lymphocytes and certain tumor cells. $DAB_{389}IL-4$ is a chimeric molecule consisting of (from the amino to the carboxy terminus) Met followed by amino acid residues 1 through 386 of mature diphtheria toxin, followed by a His-Ala dipeptide, followed by all of the amino acid residues of IL-4. Thus, $DAB_{389}IL-4$ includes all of diphtheria toxin fragment A (the enzymatically active portion of the molecule), and a

portion of fragment B. The portion of fragment B present in $DAB_{389}IL-4$ does not include the generalized receptor binding domain of diphtheria toxin, but does include the translocation domain which facilitates delivery of the enzymatically active portion into the cytosol.

Preparation of $DAB_{389}IL-4$

A synthetic gene encoding human interleukin-4 was synthesized (Milligen/Biosearch 7500 DNA synthesizer). The IL-4 sequence (Yodota et al., *Proc Nat'l Acad Sci. USA*, 83:58994, 1986) was modified to incorporate E. coli-preferred codon usage (deBoer et al., in *Maximizing Gene Expression*, Reznikioff et al., eds., 1986, Butterworths, Boston), and restriction endonuclease cleavage sites were added to facilitate subsequent cloning steps. IL-4 coding sequence (His^1 to Ser^{129}) was inserted into pSE5 plasmid (Shaw et al., J. Biol. Chem. 266:21118, 1991). The DNA sequence and corresponding amino acid sequence of this hybrid gene are shown in Fig. 1 (SEQ ID NO: 1). Following expression of $DAB_{389}IL-4$ in *E.coli*, the fusion protein was purified by standard techniques.

Alternatively, the portion of diphtheria toxin utilized in the hybrid toxin can be longer or shorter than DAB_{389}, provided that the portion used contains the enzymatically active domain and the translocation domain of diphtheria toxin, and does not contain a functional generalized eukaryotic cell-binding domain of the naturally-occurring toxin. For example, a portion containing amino acids 1 to 485 of diphtheria toxin has been incorporated into certain toxin hybrids (where the cell-binding function is supplied by a ligand such as IL-2 or α-MSH), and the resulting hybrid polypeptide has been found to intoxicate and kill cells bearing receptors for that ligand. It would therefore be expected that this 485-amino acid segment of diphtheria toxin, as well

as many others containing longer or shorter portions of fragment B sequence, would provide the necessary functions of diphtheria toxin without the undesired generalized cell-binding function of the naturally-occurring toxin molecule.

Yet another strategy for preparing the toxin portion of the hybrid would be to inactivate the receptor-binding domain of diphtheria toxin by, for example, making point mutations or internal deletions within this domain that inhibit the toxin's ability to bind to its natural receptor (Greenfield et al., *Science* 238:536, 1987).

Other Toxins

The cytotoxic portion of hybrid molecules useful in the invention can alternatively be provided by another type of toxin molecule. For example, hybrid toxins containing the enzymatically-active and translocation domains of *Pseudomonas* exotoxin A linked to IL-4 (or another IL-4R-binding ligand) can be produced by recombinant techniques in a manner analogous to that described by Chaudhary et al. (*Proc. Natl. Acad. Sci. USA* 84:4538-4542, 1987) for a *Pseudomonas* exotoxin A/TGF-α hybrid. The cell-binding regions of other toxins, including ricin, cholera toxin, *E. coli* LT, *Salmonella* LT, Shiga toxin, the Shiga-like toxins, abrin, modeccin, volkensin, and viscumin, have been shown to be located on subunits separate from those bearing the enzymatically-active or effector regions of these molecules, and so can be deleted from the toxin either by such standard means as genetic engineering or reduction of the disulfide bonds linking the subunits of a given toxin. Some of these toxins (cholera toxin and the LTs) do not typically kill the cell they intoxicate, but rather disable the cell temporarily by interfering with normal regulation of

cyclic adenosine monophosphate (cAMP) production. Thus, the use in the method of the invention of hybrid molecules that employ the effector regions of these particular toxins may be of benefit where temporary disablement, rather than killing of the target B cell, is desired.

The DNA and/or amino acid sequences corresponding to some of these naturally-occurring toxins have been published [e.g., Shiga toxin (Strockbine et al., *J. Bacteriol.* 170:1116-1122, 1988); SLT-II (Jackson et al., *FEMS Microbiol. Lett.* 44:109-114, 1987); cholera toxin (Mekalanos et al., *Nature* 306:551-557, 1983); and *E. coli* LT (Spicer and Noble, *J. Biol. Chem.* 257:5716-5721, 1982), all of which are hereby incorporated by reference], and the sequences of others can be determined by standard cloning and sequencing techniques well known to those of ordinary skill in the art.

Another source of the toxic portion of the IL-4R-targeted toxin is what is herein termed a "combination" toxin. A combination toxin is a molecule having a portion of its amino acid sequence derived from one polypeptide toxin and another portion derived from a different polypeptide toxin. The combination toxins useful in the invention would have an enzymatically active domain derived from one type of naturally-occurring toxin, a translocation domain derived from another type of toxin, and a functional cell-binding domain derived from neither; the IL-rR-binding ligand would supply the only cell-binding function of this hybrid molecule.

Naturally-occurring proteins which are known to have a translocation domain include diphtheria toxin, *Pseudomonas* exotoxin A, and possibly other peptide toxins. The translocation domains of diphtheria toxin and *Pseudomonas* exotoxin A are well characterized (see,

e.g., Hoch et al., *Proc. Natl. Acad. Sci. USA* 82:1692, 1985; Colombatti et al., *J. Biol. Chem.* 261:3030, 1986; and Deleers et al., *FEBS Lett.* 160:82, 1983), and the existence and location of such a domain in other molecules may be determined by methods such as those employed by Hwang et al. *Cell* 48:129, 1987; and Gray et al. *Proc. Natl. Acad. Sci. USA* 81:2645, 1984).

One useful IL-4/mixed toxin hybrid molecule is formed by fusing the enzymatically active A subunit of *E. coli* Shiga-like toxin (Calderwood et al., *Proc. Natl. Acad. Sci. USA* 84:4364, 1987) to a portion of fragment B of diphtheria toxin that includes a proteolytically-sensitive disulfide loop and the translocation domain (amino acid residues 186 through 386) of diphtheria toxin, and to IL-4. This three-part hybrid molecule, SLT-A/DTB'/IL-4, is useful in the method of the invention in the same way as DAB_{389}IL-4 described above. The IL-4 portion of the three-part hybrid causes the molecule to attach specifically to IL-4R-bearing cells, and the diphtheria toxin translocation portion participates in the insertion of the enzymatically-active A subunit of the Shiga-like toxin into the targeted cell. The enzymatically active portion of Shiga-like toxin, like diphtheria toxin, acts on the protein synthesis machinery of the cell to prevent protein synthesis, thus killing the cell. The difference between these two types of hybrid toxins is the nature of their enzymatic activities: the enzymatic portion of DAB_{389}IL-4 catalyzes the ADP-ribosylation by nicotinamide adenine dinucleotide of Elongation Factor 2, thereby inactivating this factor which is necessary for protein synthesis, while the enzymatic portion of SLT-A/DTB'/IL-4 is a ribonuclease capable of cleaving ribosomal RNA at a critical site, thereby inactivating the ribosome. SLT-A/DTB'/IL-4 hybrid would therefore be useful as a treatment for the

same indications as $DAB_{389}IL-4$, and could be substituted or used in conjunction with it if, for example, a patient's B cells develop a resistance to $DAB_{389}IL-4$.

Other IL-4R-binding ligands

The hybrid toxin useful in the method of the invention may employ as the IL-4R-binding ligand a moiety other than full-length IL-4. By deleting various portions of the DNA encoding IL-4 using standard genetic engineering techniques, fragments of IL-4 are generated which can be readily tested in an assay such as that described by Waters et al. (*Eur. J. Immunol.* 20:485, 1990) for their ability to bind to IL-4 receptors. Alternatively, monoclonal antibodies useful in the method of the invention can be made by immunizing mice with human $IL-4R^+$ lymphocytes (e.g., using methods similar to those of Beckmann et al., *J. Immunol.* 144:4212-4217, 1990), fusing the murine splenocytes with appropriate myeloma cells, and screening the antibodies produced by the resultant hybridoma lines for the requisite IL-4R binding properties by, for example, assaying their ability to inhibit ^{125}I-labeled IL-4 binding to $IL-4R^+$ cells using the method of Mosley et al., *Cell* 59:335-348, 1989. Alternatively, useful antibodies may be isolated from a combinatorial library produced by the method of Huse et al. (*Science* 246:1275, 1989).

The invention can employ not only intact monoclonal antibodies as the IL-4R-binding ligand, but also an immunologically-active antibody fragment, for example, a Fab or $(Fab)_2$ fragment; an antibody heavy chain; an antibody light chain; a genetically engineered single-chain Fv molecule (Ladner et al., U.S. Patent No. 4,946,778); or a chimeric antibody, for example, an antibody which contains the binding specificity of a murine antibody, but in which the remaining portions are

of human origin, or an antibody whose Fv region is genetically engineered to capture the higher binding affinity of its target receptor's natural targeting ligand.

Linkage of Toxins to Binding Ligands

The binding ligand and the cytotoxin of useful hybrid molecules can be linked in several ways. If the hybrid molecule is produced by expression of a fused gene, a peptide bond serves as the link between the cytotoxin and the binding ligand. Alternatively, the toxin and the binding ligand can be produced separately and later coupled by means of a non-peptide covalent bond, such as a disulfide bond. In this case, if the binding ligand is a protein, e.g., IL-4, the DNA encoding IL-4 can be engineered to contain an extra cysteine codon in a manner analogous to that described in Murphy et al. U.S. Serial No. 313,599, hereby incorporated by reference. The cysteine must be positioned so as to not interfere with the IL-4R binding activity of the hybrid molecule. For example, the cysteine codon can be inserted just upstream of the DNA encoding the mature form of IL-4. The toxin molecule must be derivatized with a sulfhydryl group reactive with the cysteine on the modified IL-4. In the case of a peptide toxin, this can be accomplished by inserting an extra cysteine codon into the DNA sequence encoding the toxin. Alternatively, a sulfhydryl group, either by itself or as part of a cysteine residue, can be introduced using known synthetic techniques. For example, the introduction of sulfhydryl groups into peptides is described by Hiskey (*Peptides* 3:137, 1981). Derivatization can also be carried out according to the method described for the derivatization of a peptide hormone in Bacha et al. U.S. Patent No. 4,468,382, hereby incorporated by reference. The

introduction of sulfhydryl groups into proteins is described in Maasen et al. (*Eur. J. Biochem.* 134:32, 1983). Once the correct sulfhydryl groups are present, the cytotoxin and IL-4R-binding ligand are separately purified; both sulfur groups are reduced; cytotoxin and ligand are mixed (in a ratio of about 1:5 to 1:20); and disulfide bond formation is allowed to proceed to completion (generally 20 to 30 minutes) at room temperature. The mixture is then dialyzed against phosphate buffered saline to remove unreacted ligand and toxin molecules. Sephadex chromatography or the like is then carried out to separate on the basis of size the desired toxin-ligand conjugates from toxin-toxin and ligand-ligand conjugates.

Assays for IL-4 Receptor Binding

The IL-4R binding activity of various molecules can be measured using the assay described by Park et al. (*J. Exp. Med.* 166:476, 1987) or the assay described by Foxwell et al. (*Eur. J. Immunol.* 19:1637, 1989).

Assays for Toxicity

Toxicity towards IL-4R bearing cells in general can be tested as follows. Cultured HUT 102/6TG cells (Tsudo et al., *Proc. Natl. Acad. Sci. USA* 83:9694, 1986) or MLA144 cells (Rabin et al. *J. Immunol.* 127:1852, 1981) are maintained in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 25 mM HEPES (pH 7.4), 2mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum (Hazelton, Lenexa, KS). Cells are seeded in 96-well V-bottomed plates (Linbro-Flow Laboratories, McLean, VA) at a concentration of 1×10^5 per well in complete medium. Putative toxins are added at varying concentrations (10^{-12}M to 10^{-6}M) and the cultures are incubated for 20 hrs. at 37°C in a 5% CO_2

atmosphere. Following incubation, the plates are centrifuged for 5 min. at 170 x g, and the medium removed and replaced with 100 μl leucine-free medium (MEM, Gibco) containing 8 μCi/ml (^{3}H-leucine; New England Nuclear, Boston, MA). After an additional 90 min. at 37°C, the plates are centrifuged for 5 min. at 170 x g, the medium is removed, and the cells are collected on glass fiber filters using a cell harvester (Skatron, Sterling, VA). Filters are washed, dried, and counted according to standard methods. Cells cultured with medium alone serve as the control. Effective cell killing is indicated by a decrease in ^{3}H-leucine incorporation in test samples, compared to control samples which do not contain the toxin.

Assay for ability to prevent class-switching of B cells

Materials and Methods

Interleukins and Antibodies. Human rIL-4 was used in a purified form (specific activity: 1.2 x 10^7 U/mg). Anti-Leu-4 (IgG1 anti-CD3), anti-Leu-3a (IgG1 anti-CD4), and Leu-2a (IgG1 anti-CD8), as well as the appropriate isotype controls, were obtained from Becton Dickinson & Co. (Mountain View, CA). $F(ab^1)_2$ fragments of monoclonal antibody 626.1 (IgG_1 anti-CD40) were obtained as described in Gruber et al., J. Immuno. 142:4144 (1989). OKT3 (IgG2a anti-CD3) mAb was obtained from Ortho Diagnostic Systems Inc. (Westwood, MA). mAb B1 (IgG2a anti-CD20) was obtained from Coulter Immunology (Hialeah, FL).

Cell Preparations. PBMC were isolated from heparinized venous blood of normal nonallergic donors by density gradient centrifugation on Ficoll-Hypaque, washed three times in HBSS (Microbiological Associates, Bethesda, MD) and resuspended in RPMI 1640/10% heat inactivated FCS (HyClone Laboratories, Logan, UT)

supplemented with 2 mM L-glutamine, 50 μg/ml streptomycin and 100 U/ml penicillin (complete medium). To obtain purified B cells, T cells were removed by rosetting twice with 2-aminoethylisothiouronium bromide (AET)-treated SRBC. Further T cell depletion was obtained by two cycles of lysis with anti-CD3 mAb + rabbit C (Pel-Freeze Biologicals, Inc., Rogers, AR). To remove monocytes, non-T cells in RPMI 1640/10% AB^+ serum were adhered twice in plastic petri dishes. The resulting B cell populations contained <6% $CD14^+$ cells and <1% $CD3^+$ cells, as determined by immunofluorescence (IF). In addition, these B cell preparations gave no proliferative response to Con A or PHA (10 μg/ml), while they strongly proliferated upon stimulation with PMA (25 ng/ml; Sigma Chemical Co., St. Louis, MO) and insolubilized anti-μ antibody (Immunobead rabbit anti-human IgM; 1μg/ml; Bio-Rad Laboratories, Richmond, CA). Cell viability, as assessed by trypan blue exclusion, was always >95%.

Cell Cultures for IgE Induction. Purified B cells (1.0×10^6 cells/ml) in complete medium were cultured at 37°C in a 5% CO_2 humidified atmosphere, in the presence of rIL-4 (100 U/ml) and the various mAbs, as indicated for each experiment in Results. After 10 d, the culture supernatants were harvested and assessed by RIA for their IgE content. Control cultures for the evaluation of preformed IgE were set up in the presence of cycloheximide (100 μg/ml; Sigma Chemical Co.). Net IgE synthesis was evaluated by subtracting the IgE concentrations detected in cycloheximide-treated cultures from the IgE values found in untreated cultures.

RIA for IgE. The assay was performed in flexible flat-bottomed microtiter plates (Cooke Laboratory Products, Alexandria, VA) at room temperature as previously described (3). The wells were coated with 0.1 ml of a 1:1 mixture of purified anti-Fcε mAbs (7.12 and

4.15; a kind gift of A. Saxon, University of California Los Angeles, Los Angeles, CA), 2 μg/ml in carbonate-bicarbonate buffer, pH 9.6. After 16-h incubation, the wells were washed, blocked with PBS/10% horse serum (HS) for 2 h, and subsequently washed three times with PBS/1% HS. 0.1 ml of culture supernatant or different dilutions of IgE standard (Pharmacia Fine Chemicals) were then added to the wells in triplicate and incubated for 16 h in a humidified chamber. The wells were then washed one time with PBS/1% HS containing 0.05% Tween 20, twice with PBS/1% HS, and finally incubated with 0.1 ml of Phadebas RAST ^{125}I-anti-human IgE (ND) (Pharmacia Fine Chemicals) for 6 h. The wells were then washed three times with PBS/1% HS/0.05% Tween 20 and eight times under running distilled water, cut out, and counted in a gamma spectrometer (Tracor Analytic, Elk Grove Village, IL). The concentrations of IgE in the supernatants were read from the standard curve. The lower limit of sensitivity of this assay is 150 pg/ml. This assay was validated in a recent multicenter collaborative assessment of the variability of IgE measurement in cell culture supernatants [Helm et al., J. Allergy Clin. Immunol. 77:880 (1986)].

Results

In the model system, purified surface-IgE-negative B cells undergo class switch to IgE production if and only if both rIL-4 and anti-CD40 monoclonal antibody are included in the cultures. When cultured alone or in the presence of <u>either</u> rIL-4 <u>or</u> anti-CD40 monoclonal antibody, these B cells fail to produce IgE. As shown in Table I, the addition of increasing concentrations of DAB_{389}IL-4 ablated the IgE response in these cultures in a dose-dependent manner. As long as DAB_{389}IL-4 was present at the initiation of the culture, IgE synthesis was inhibited in the presence of rIL-4 and anti-CD40

monoclonal antibody regardless of their order of addition.

Table II shows that purified B cells from an atopic donor which have already undergone an Ig class switch to IgE production require neither rIL-4 nor anti-CD40 monoclonal antibody to maintain IgE production. Furthermore, $DAB_{389}IL-4$ was unable significantly to inhibit IgE production in cultures in which the class switch had already occurred, in contrast to the marked effect the hybrid toxin has on previously unswitched cells.

Table I

DAB_{389}IL-4 Elimates IgE Secretion by B cells Undergoing Ig Class Switching

Culture conditions		IgE (pg/ml)
B cells alone (1 x 10^6ml)		2
rIL-4 (100 U/ml)		2
CD40 mAb (F[ab]'$_2$, 5 μg/ml)		ND
A. DAB_{389}IL-4 for 24h, wash and add αCD40 mAb+rIL-4		
1.	DAB_{389}IL-4 (10^{-7}M)	1
2.	(10^{-8}M)	2
3.	(10^{-9}M)	2
4.	(10^{-10}M)	421
5.	Medium	398
B. DAB_{389}IL-4 and αCD40 mAb for 24h, then add rIL-4		
1.	DAB_{389}IL-4 (10^{-7}M)	2
2.	(10^{-8}M)	2
3.	(10^{-9}M)	3
4.	(10^{-10}M)	3980
5.	Medium	2584
C. DAB_{389}IL-4, αCD40 mAb and rIL-4 added at beginning of culture		
1.	DAB_{389}IL-4 (10^{-7}M)	2
2.	(10^{-8}M)	2
3.	(10^{-9}M)	3
4.	(10^{-10}M)	169
5.	Medium	1372

Table II

DAB_{389}IL-4 Does Not Elimate IgE Secretion by B cells from an Atopic Patient Which Hav Already Undergone an Ig Class Switch

Culture conditions		IgE (pg/ml)
B cells alone (1 x 10^6ml)		34,446
rIL-4 (100 U/ml)		30,338
CD40 mAb (F[ab]'$_2$, 5 μg/ml)		29,119
A. DAB_{389}IL-4 for 24h, wash and add αCD40 mAb+rIL-4		
1.	DAB_{389}IL-4 (10^{-7}M)	20,188
2.		(10^{-8}M) 27,646
3.		(10^{-9}M) 30,470
4.		(10^{-10}M) 28,659
5.	Medium	28,078
B. DAB_{389}IL-4 and αCD40 mAb for 24h, then add rIL-4		
1.	DAB_{389}IL-4 (10^{-7}M)	14,064
2.		(10^{-8}M) 34,237
3.		(10^{-9}M) 37,506
4.		(10^{-10}M) 22,889
5.	Medium	21,353
C. DAB_{389}IL-4, αCD40 mAb, rIL-4 added at beginning of culture		
1.	DAB_{389}IL-4 (10^{-7}M)	14,127
2.		(10^{-8}M) 39,436
3.		(10^{-9}M) 32,052
4.		(10^{-10}M) 27,290
5.	Medium	29,929

Animal models

The ability of a particular hybrid IL-4R-binding toxin to diminish or ablate IgE production *in vivo* can be studied in an animal model such as the mouse assay utilized by Urban et al. (*Proc. Natl. Acad. Sci. USA* 88:5513-5517, 1991), or in a species (such as dogs) known to develop allergic responses to certain allergens.

Therapy

Desensitization with the method of the invention will probably be most effective if carried out on naive subjects, or on atopic subjects who are not currently mounting an allergic response. A typical protocol would involve exposing the subject animal to one or more allergens of interest (e.g., by intravenous injection) simultaneously with the i.v. administration of the IL-4R-specific toxin on day 1, followed by four more days of treatment once per day with the IL-4R-specific toxin alone. It is expected that a dosage regimen which produces a serum concentration of about 10^{-10} to 10^{-7}M $DAB_{389}IL-4$ (preferably 10^{-9} to 10^{-8}M) will effectively kill most allergen-activated B cells about to undergo an isotype class shift, without significant harm to those cells which have fewer or no IL-4 receptors. This course of treatment can be repeated several times to provide effective therapy. Determination of the most efficacious treatment protocol for desensitizing a subject to a particular allergen or group of allergens using the method of the invention is within the ability of one of ordinary skill in pharmacology, using the disclosure provided herein and standard pharmacological procedures.

Other embodiments are within the following claims.

What is claimed is:

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Seragen, Inc.

(ii) TITLE OF INVENTION: Desensitization to Specific Allergens

(iii) NUMBER OF SEQUENCES: 1

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Fish & Richardson
(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: U.S.A.
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM: IBM P.C. DOS (Version 3.30)
(D) SOFTWARE: WordPerfect (Version 5.0)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 07/832,843
(B) FILING DATE: 10 February 1992

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Fraser, Janis K.
(B) REGISTRATION NUMBER: 34,819
(C) REFERENCE/DOCKET NUMBER: 00563/055WO1

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617) 542-5070
(B) TELEFAX: (617) 542-8906
(C) TELEX: 200154

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1604
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Met Gly Ala Asp Asp Val Val Asp Ser Ser Lys Ser Phe Val Met Glu
1               5                  10                  15

AAC TTT TCT TCG TAC CAC GGG ACT AAA CCT GGT TAT GTA GAT TCC ATT   96
Asn Phe Ser Ser Tyr His Gly Thr Lys Pro Gly Tyr Val Asp Ser Ile
            20                  25                  30

CAA AAA GGT ATA CAA AAG CCA AAA TCT GGT ACA CAA GGA AAT TAT GAC  144
Gln Lys Gly Ile Gln Lys Pro Lys Ser Gly Thr Gln Gly Asn Tyr Asp
        35                  40                  45

GAT GAT TGG AAA GGG TTT TAT AGT ACC GAC AAT AAA TAC GAC GCT GCG  192
Asp Asp Trp Lys Gly Phe Tyr Ser Thr Asp Asn Lys Tyr Asp Ala Ala
    50                  55                  60

GGG TAC TCT GTA GAT AAT GAA AAC CCG CTC TCT GGA AAA GCT GGA GGC  240
Gly Tyr Ser Val Asp Asn Glu Asn Pro Leu Ser Gly Lys Ala Gly Gly
65                  70                  75                  80

GTG GTC AAA GTG ACG TAT CCA GGA CTG ACG AAG GTT CTC GCA CTA AAA  288
Val Val Lys Val Thr Tyr Pro Gly Leu Thr Lys Val Leu Ala Leu Lys
                85                  90                  95

GTG GAT AAT GCC GAA ACT ATT AAG AAA GAG TTA GGT TTA AGT CTC ACT  336
Asp Asn Ala Glu Thr Ile Lys Lys Glu Leu Gly Leu Ser Leu Thr Glu
            100                 105                 110

GAA CCG TTG ATG GAG CAA GTC GGA ACG GAA GAG TTT ATC AAA AGG TTC  384
Val Pro Leu Met Glu Gln Val Gly Thr Glu Glu Phe Ile Lys Arg Phe
        115                 120                 125

GGT GAT GGT GCT TCG CGT GTA GTG CTC AGC CTT CCC TTC GCT GAG GGG  432
Gly Asp Gly Ala Ser Arg Val Val Leu Ser Leu Pro Phe Ala Glu Gly
    130                 135                 140

AGT TCT AGC GTT GAA TAT ATT AAT AAC TGG GAA CAG GCG AAA GCG TTA  480
Ser Ser Ser Val Glu Tyr Ile Asn Asn Trp Glu Gln Ala Lys Ala Leu
145                 150                 155                 160

AGC GTA GAA CTT GAG ATT AAT TTT GAA ACC CGT GGA AAA CGT GGC CAA  528
Ser Val Glu Leu Glu Ile Asn Phe Glu Thr Arg Gly Lys Arg Gly Gln
                165                 170                 175
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GAT GCG ATG TAT GAG TAT ATG GCT CAA GCC TGT GCA GGA AAT CGT GTC  576
Asp Ala Met Tyr Glu Tyr Met Ala Gln Ala Cys Ala Gly Asn Arg Val
            180                 185                 190

AGG CGA TCA GTA GGT AGC TCA TTG TCA TGC ATA AAT CTT GAT TGG GAT  624
Arg Arg Ser Val Gly Ser Ser Leu Ser Cys Ile Asn Leu Asp Trp Asp
        195                 200                 205

GTC ATA AGG GAT AAA ACT AAG ACA AAG ATA GAG TCT TTG AAA GAG CAT  672
Val Ile Arg Asp Lys Thr Lys Thr Lys Ile Glu Ser Leu Lys Glu His
    210                 215                 220

GGC CCT ATC AAA AAT AAA ATG AGC GAA AGT CCC AAT AAA ACA GTA TCT  720
Gly Pro Ile Lys Asn Lys Met Ser Glu Ser Pro Asn Lys Thr Val Ser
225                 230                 235                 240

GAG GAA AAA GCT AAA CAA TAC CTA GAA GAA TTT CAT CAA ACG GCA TTA  768
Glu Glu Lys Ala Lys Gln Tyr Leu Glu Glu Phe His Gln Thr Ala Leu
                245                 250                 255

GAG CAT CCT GAA TTG TCA GAA CTT AAA ACC GTT ACT GGG ACC AAT CCT  816
Glu His Pro Glu Leu Ser Glu Leu Lys Thr Val Thr Gly Thr Asn Pro
            260                 265                 270

GTA TTC GCT GGG GCT AAC TAT GCG GCG TGG GCA GTA AAC GTT GCG CAA  864
Val Phe Ala Gly Ala Asn Tyr Ala Ala Trp Ala Val Asn Val Ala Gln
        275                 280                 285

GTT ATC GAT AGC GAA ACA GCT GAT AAT TTG GAA AAG ACA ACT GCT GCT  912
Val Ile Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys Thr Thr Ala Ala
    290                 295                 300

CTT TCG ATA CTT CCT GGT ATC GGT AGC GTA ATG GGC AAT GCA GAC GGT  960
Leu Ser Ile Leu Pro Gly Ile Gly Ser Val Met Gly Asn Ala Asp Gly
305                 310                 315                 320

GCC GTT CAC CAC AAT ACA GAA GAG ATA GTG GCA CAA TCA ATA GCT TTA 1008
Ala Val His His Asn Thr Glu Glu Ile Val Ala Gln Ser Ile Ala Leu
                325                 330                 335

TCG TCT TTA ATG GTT GCT CAA GCT ATT CCA TTG GTA GGA GAG CTA GTT 1056
Ser Ser Leu Met Val Ala Gln Ala Ile Pro Leu Val Gly Glu Leu Val
            340                 345                 350
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GAT ATT GGT TTC GCT GCA TAT AAT TTT GTA GAG AGT ATT ATC AAT TTA 1104
Asp Ile Gly Phe Ala Ala Tyr Asn Phe Val Glu Ser Ile Ile Asn Leu
        355                 360                 365

TTT CAA GTA GTT CAT AAT TCG TAT AAT CGT CCC GCG TAT TCT CCG GGT 1152
Phe Gln Val Val His Asn Ser Tyr Asn Arg Pro Ala Tyr Ser Pro Gly
    370                 375                 380

CAC AAA ACG CAT GCT CAC AAA TGC GAC ATC ACC CTG CAG GAA ATC ATC 1200
His Lys Thr His Ala His Lys Cys Asp Ile Thr Leu Gln Glu Ile Ile
385                 390                 395                 400

AAA ACT CTG AAT TCC CTG ACC GAA CAG AAA ACT CTG TGC ACC GAA CTG 1248
Lys Thr Leu Asn Ser Leu Thr Glu Gln Lys Thr Leu Cys Thr Glu Leu
                405                 410                 415

ACG GTA ACC GAC ATC TTC GCT GCA TCC AAA AAC ACC ACT GAA AAA GAA 1296
Thr Val Thr Asp Ile Phe Ala Ala Ser Lys Asn Thr Thr Glu Lys Glu
            420                 425                 430

ACC TTC TGC CGT GCA GCA ACT GTT CTG CGT CAG TTC TAC TCC CAC CAC 1344
Thr Phe Cys Arg Ala Ala Thr Val Leu Arg Gln Phe Tyr Ser His His
        435                 440                 445

GAA AAA GAC ACT CGC TGC CTT GGT GCT ACT GCA CAG CAG TTC CAC CGT 1392
Glu Lys Asp Thr Arg Cys Leu Gly Ala Thr Ala Gln Gln Phe His Arg
    450                 455                 460

CAC AAA CAG CTG ATC CGT TTC CTG AAA CGT CTA GAC CGC AAC CTG TGG 1440
His Lys Gln Leu Ile Arg Phe Leu Lys Arg Leu Asp Arg Asn Leu Trp
465                 470                 475                 480

GGC CTG GCT GGC CTG AAC TCC TGT CCG GTT AAA GAA GCT AAC CAG TCG 1488
Gly Leu Ala Gly Leu Asn Ser Cys Pro Val Lys Glu Ala Asn Gln Ser
                485                 490                 495

ACC CTG GAA AAC TTC CTG GAA CGT CTG AAA ACC ATC ATG CGT GAA AAA 1536
Thr Leu Glu Asn Phe Leu Glu Arg Leu Lys Thr Ile Met Arg Glu Lys
            500                 505                 510

TAC TCT AAA TGT TCT TCC TGAGAGCTCA GTACTAGCCC GCCTAATGAG        1584
Tyr Ser Lys Cys Ser Ser
        515

CGGGCTTTTT TTTAGGCCTA                                          1604
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Claims

1. Use of a molecule which a) specifically binds under physiological conditions to an interleukin-4 (IL-4) receptor expressed on a peripheral blood mononuclear cell (PBMC) of an animal, and b) is capable of decreasing the viability of said PBMC in the preparation of a medicament for desensitizing an animal to an allergen.

2. The use of claim 1, wherein said molecule kills said PBMC following binding to said IL-4 receptor.

3. The use of claim 1, wherein said molecule is a hybrid molecule comprising a first and a second portion joined together covalently, said first portion comprising a moiety capable of decreasing cell viability and said second portion comprising a moiety capable of specifically binding to said IL-4 receptor under physiological conditions.

4. The use of claim 3, wherein said second portion comprises all or a binding portion of an antibody specific for said IL-4 receptor.

5. The use of claim 3, wherein said second portion comprises all or a binding portion of IL-4.

6. The use of claim 3, wherein said first portion comprises an enzymatically-active segment of a polypeptide toxin.

7. The use of claim 6, wherein said polypeptide toxin is diphtheria toxin, *Pseudomonas* exotoxin A, ricin, Shiga toxin, Shiga-like toxin-I, Shiga-like toxin II, Shiga-like toxin II_v, *E. coli* LT, *Salmonella* LT, cholera toxin, C3 toxin, pertussis toxin, tetanus toxin, abrin, modeccin, volkensin, viscumin, alorin, saporin, or gelonin.

8. The use of claim 7, wherein said polypeptide toxin is diphtheria toxin.

9. The use of claim 6, wherein said segment does not possess a generalized eukaryotic cell-binding activity.

10. The use of claim 9, wherein said segment comprises fragment A of diphtheria toxin and a portion of fragment B of diphtheria toxin.

11. The use of claim 10, wherein said segment comprises DAB_{389}.

12. The use of claim 11, wherein said molecule is DAB_{389}IL-4.

13. The use of claim 10, wherein said second portion comprises an IL-4-receptor-binding portion of an antibody specific for said IL-4 receptor.

14. Use of a molecule which is capable of specifically binding to an IL-4 receptor expressed on the surface of a B cell, and which is capable of decreasing the viability of said B cell in the preparation of a

medicament for inhibiting the antibody heavy-chain class switching of a B cell.

15. The use of claim 14, wherein said molecule is a hybrid molecule comprising a first and a second portion joined together covalently, said first portion comprising a moiety capable of decreasing cell viability and said second portion comprising a moiety capable of specifically binding to said IL-4 receptor under physiological conditions.

16. The use of claim 15, wherein said second portion comprises all or a binding portion of an antibody specific for said IL-4 receptor.

17. The use of claim 15, wherein said second portion comprises all or a binding portion of IL-4.

18. The use of claim 15, wherein said first portion comprises an enzymatically-active segment of a polypeptide toxin.

19. The use of claim 18, wherein said polypeptide toxin is diphtheria toxin, *Pseudomonas* exotoxin A, ricin, Shiga toxin, Shiga-like toxin-I, Shiga-like toxin II, Shiga-like toxin II_v, *E. coli* LT, *Salmonella* LT, cholera toxin, C3 toxin, pertussis toxin, tetanus toxin, abrin, modeccin, volkensin, viscumin, alorin, saporin, or gelonin.

20. The use of claim 19, wherein said polypeptide toxin is diphtheria toxin.

21. The use of claim 20, wherein said segment comprises fragment A of diphtheria toxin and a portion of fragment B of diphtheria toxin.

22. The use of claim 21, wherein said segment comprises DAB_{389}.

23. The use of claim 22, wherein said molecule is DAB_{389}IL-4.

24. The use of claim 1, further comprising the use of a second molecule which a) specifically binds under physiological conditions to an interleukin-2 (IL-2) receptor expressed on said PBMC or a second PBMC of said animal, and b) is capable of decreasing the viability of the PBMC to which it binds in the preparation of said medicament.

25. The use of claim 1, further comprising the use of a second molecule which a) specifically binds under physiological conditions to an interleukin-6 (IL-6) receptor expressed on said PBMC or a second PBMC of said animal, and b) is capable of decreasing the viability of the PBMC to which it binds in the preparation of said medicament.

```
                10          20          30          40
                |           |           |           |
ATG GGC GCT GAT GAT GTT GTT GAT TCT TCT AAA TCT TTT GTG ATG

MET Gly Ala Asp Asp Val Val Asp Ser Ser Lys Ser Phe Val MET

     50          60              70          80          90
     |           |               |           |           |
GAA AAC TTT TCT TCG TAC CAC GGG ACT AAA CCT GGT TAT GTA GAT
Glu Asn Phe Ser Ser Tyr His Gly Thr Lys Pro Gly Tyr Val Asp

           100         110         120             130
            |           |           |               |
TCC ATT CAA AAA GGT ATA CAA AAG CCA AAA TCT GGT ACA CAA GGA
Ser Ile Gln Lys Gly Ile Gln Lys Pro Lys Ser Gly Thr Gln Gly

   140         150             160         170         180
    |           |               |           |           |
AAT TAT GAC GAT GAT TGG AAA GGG TTT TAT AGT ACC GAC AAT AAA
Asn Tyr Asp Asp Asp Trp Lys Gly Phe Tyr Ser Thr Asp Asn Lys
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FIG. 1

(PAGE 1 OF 8)

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                190       200       210       220
                 |         |         |         |
TAC GAC GCT GCG GGG TAC TCT GTA GAT AAT GAA AAC CCG CTC TCT
Tyr Asp Ala Ala Gly Tyr Ser Val Asp Asn Glu Asn Pro Leu Ser

        230       240       250       260       270
         |         |         |         |         |
GGA AAA GCT GGA GGC GTG GTC AAA GTG ACG TAT CCA GGA CTG ACG
Gly Lys Ala Gly Gly Val Val Lys Val Thr Tyr Pro Gly Leu Thr

                280       290       300       310
                 |         |         |         |
AAG GTT CTC GCA CTA AAA GTG GAT AAT GCC GAA ACT ATT AAG AAA
Lys Val Leu Ala Leu Lys Asp Asn Ala Glu Thr Ile Lys Lys Lys
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FIG. 1
(PAGE 2 OF 8)

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   320          330          340          350          360
GAG TTA GGT TTA AGT CTC ACT GAA CCG TTG ATG GAG CAA GTC GGA
Glu Leu Gly Leu Ser Leu Thr Glu Pro Leu MET Glu Gln Val Gly

         370          380          390          400
ACG GAA GAG TTT ATC AAA AGG TTC GGT GAT GGT GCT TCG CGT GTA
Thr Glu Glu Phe Ile Lys Arg Phe Gly Asp Gly Ala Ser Arg Val

   410          420          430          440          450
GTG CTC AGC CTT CCC TTC GCT GAG GGG AGT TCT AGC GTT GAA TAT
Val Leu Ser Leu Pro Phe Ala Glu Gly Ser Ser Ser Val Glu Tyr

         460          470          480          490
ATT AAT AAC TGG GAA CAG GCG AAA GCG TTA AGC GTA GAA CTT GAG
Ile Asn Asn Trp Glu Gln Ala Lys Ala Leu Ser Val Glu Leu Glu

   500          510          520          530          540
ATT AAT TTT GAA ACC CGT GGA AAA CGT GGC CAA GAT GCG ATG TAT
Ile Asn Phe Glu Thr Arg Gly Lys Arg Gly Gln Asp Ala MET Tyr
```

FIG. 1
(PAGE 3 OF 8)

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          550         560         570         580
GAG TAT ATG GCT CAA GCC TGT GCA GGA AAT CGT GTC AGG CGA TCA
Glu Tyr MET Ala Gln Ala Cys Ala Gly Asn Arg Val Arg Arg Ser

   590         600         610         620         630
GTA GGT AGC TCA TTG TCA TGC ATA AAT CTT GAT TGG GAT GTC ATA
Val Gly Ser Ser Leu Ser Cys Ile Asn Leu Asp Trp Asp Val Ile

          640         650         660         670
AGG GAT AAA ACT AAG ACA AAG ATA GAG TCT TTG AAA GAG CAT GGC
Arg Asp Lys Thr Lys Thr Lys Ile Glu Ser Leu Lys Glu His Gly

   680         690         700         710         720
CCT ATC AAA AAT AAA ATG AGC GAA AGT CCC AAT AAA ACA GTA TCT
Pro Ile Lys Asn Lys MET Ser Glu Ser Pro Asn Lys Thr Val Ser
```

FIG. 1

(PAGE 4 OF 8)

```
          730               740               750               760
GAG GAA AAA GCT AAA CAA TAC CTA GAA GAA TTT CAT CAA ACG GCA
Glu Glu Lys Ala Lys Gln Tyr Leu Glu Glu Phe His Gln Thr Ala

   770               780               790               800               810
TTA GAG CAT CCT GAA TTG TCA GAA CTT AAA ACC GTT ACT GGG ACC
Leu Glu His Pro Glu Leu Ser Glu Leu Lys Thr Val Thr Gly Thr

          820               830               840               850
AAT CCT GTA TTC GCT GGG GCT AAC TAT GCG GCG TGG GCA GTA AAC
Phe Ala Gly Ala Asn Tyr Ala Ala Trp Ala Val Asn Val Ala Gln

   860               870               880               890               900
GTT GCG CAA GTT ATC GAT AGC GAA ACA GCT GAT AAT TTG GAA AAG
Asn Pro Val Val Ile Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys

          910               920               930               940
ACA ACT GCT GCT CTT TCG ATA CTT CCT GGT ATC GGT AGC GTA ATG
Thr Thr Ala Ala Leu Ser Ile Leu Pro Gly Ile Gly Ser Val MET
```

FIG. 1
(PAGE 5 OF 8)

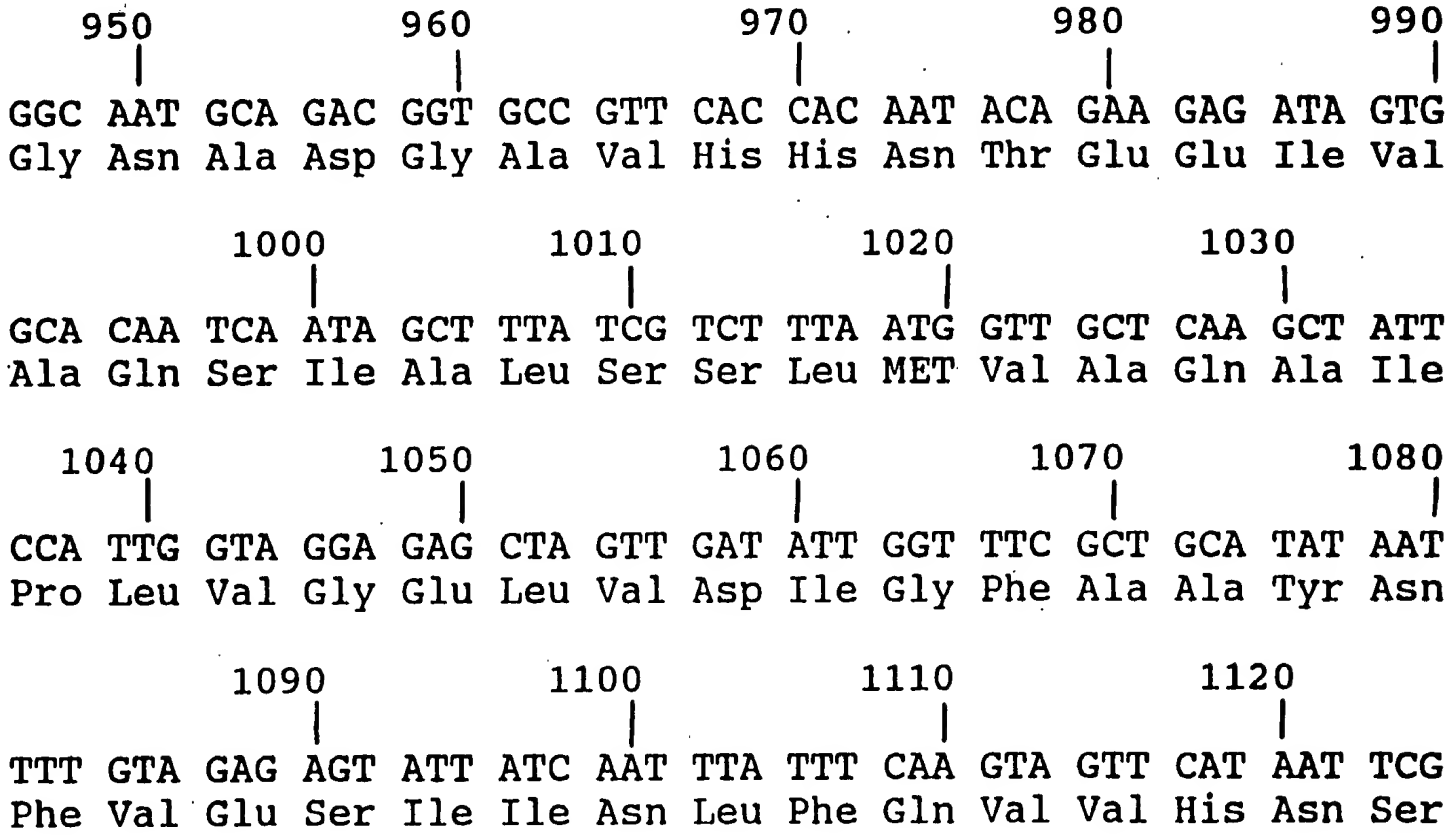

FIG. 1
(PAGE 6 OF 8)

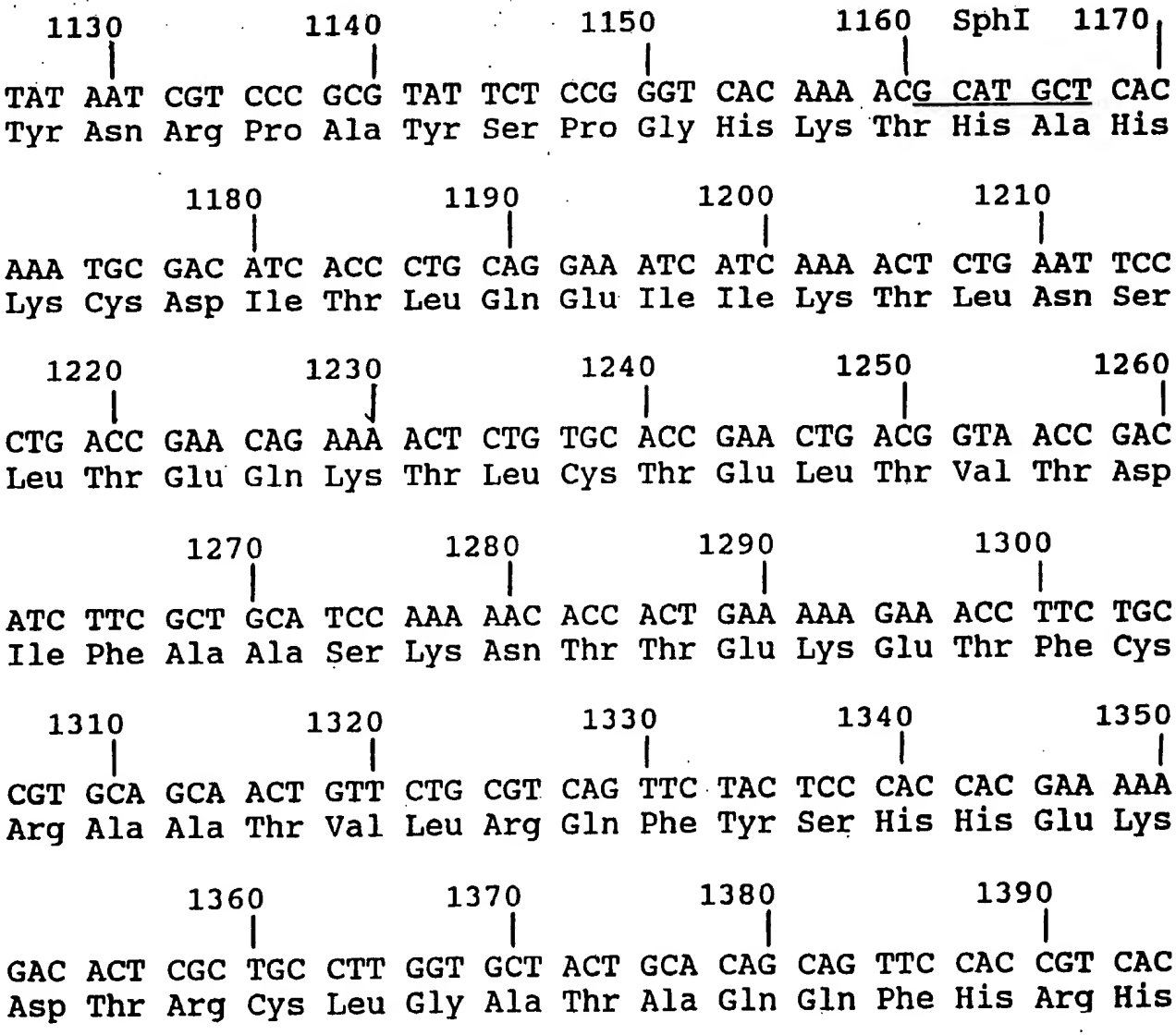

FIG. 1
(PAGE 7 OF 8)

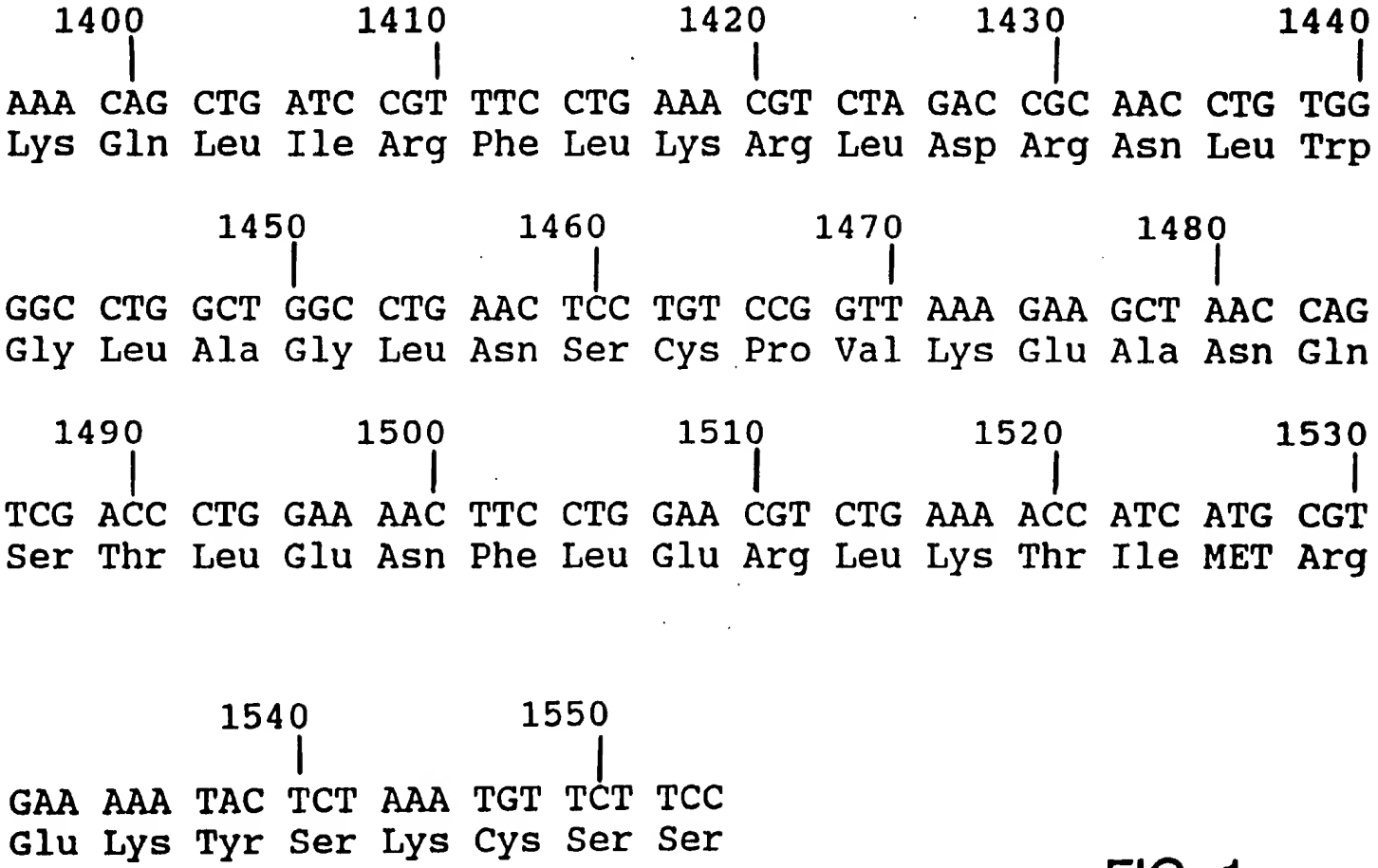

FIG. 1
(PAGE 8 OF 8)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01034

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 45/05, 39/00, 37/02
US CL :424/85.1, 85.2, 85.8, 88; 514/2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.1, 85.2, 85.8, 88; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DATABASES: US PTO-PAS, Medline
SEARCH TERMS: Interleukin-4, -6; Allergy; IgM, Immunoglobulin, Switch

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings of the National Academy of Sciences of the USA, Volume 86, issued June 1989, M. Ogata et al., "Cytotoxic activity of a recombinant fusion protein between interleukin 4 and Pseudomonas exotoxin", pages 4215-19, especially pages 4215 and 4219.	1-25
Y	European Journal of Immunology, Volume 20, issued 1990, J. C. Prinz et al., "Allergen-directed expression of Fc receptors for IgE (CD23) on human T lymphocytes is modulated by interleukin 4 and interferon-gamma", pages 1259-64, especially pages 1259 and 1263.	1-13, 24, 25

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search 19 April 1993	Date of mailing of the international search report 30 APR 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer DAVID L. FITZGERALD Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01034

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EMBO Journal, Volume 8, Number 2, issued 1989, C. Esser et al., "Rapid induction of transcription of unrearranged s-gamma 1 switch regions in activated murine B cells by interleukin 4", pages 483-88, especially the abstract.	14-23
Y	Kidney International, Volume 35, issued 1989, T. B. Strom et al., "Toward more selective therapies to block undesired immune responses", pages 1026-33, see the entire document.	24, 25
Y	Proceedings of the National Academy of Sciences of the USA, Volume 85, issued December 1988, C. B. Siegall et al., "Cytotoxic activity of an interleukin 6-Pseudomonas exotoxin fusion protein on human myeloma cells", pages 9738-42, especially the abstract.	25